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(54) Title: **METHOD AND APPARATUS FOR SELECTIVE BIOLOGICAL MATERIAL DETECTION**

(57) **Abstract:** The present invention relates to bioassay materials useful for the detection of toxic substances and, more particularly, to packaging materials for food and other products, along with methods for their manufacture and use. The invention provides a unique composite material capable of detecting and identifying multiple biological material within a single package. The biological material identification system is designed for incorporation into existing types of flexible packaging material such as polyvinylchloride or polyolefin films, and its introduction into the existing packaging infrastructure will require little or no change to present systems and procedures.

1 Method and Apparatus for Selective Biological Material
2 Detection

3
4 Reference to Related Applications:

5 This application is a continuation-in-part of S.N.
6 09/218,827, filed on Dec. 22, 1998 and now U.S. Patent
7 6,051,388, having an issue date of April 18, 2000, the
8 contents of which are herein incorporated by reference.

9
10 Field of the Invention

11 This invention relates to the detection of pathogenic
12 microorganisms, or biological materials, and more
13 particularly relates to a composite bioassay material
14 useful for the detection of particular toxic substances,
15 its method of manufacture and method of use, wherein the
16 composite material is particularly useful for food
17 packaging and the like, and is capable of simultaneously
18 detecting and identifying a multiplicity of such
19 biological materials.

20

21 Background of the Invention

22 Although considerable effort and expense have been
23 put forth in an effort to control food borne pathogenic
24 microorganisms, there nevertheless exist significant
25 safety problems in the supply of packaged food. For
26 example, numerous outbreaks of food poisoning brought
27 about by foodstuffs contaminated with strains of the E-
28 Coli, Campylobacter, Listeria, Cyclospora and Salmonella
29 microorganisms have caused illness and even death, not to
30 mention a tremendous loss of revenue for food producers.
31 These and other microorganisms can inadvertently taint
32 food, even when reasonably careful food handling
33 procedures are followed. The possibility of accidental
34 contamination, for example by temperature abuse, in and of
35 itself, is enough to warrant incorporation of safe and

1 effective biological material diagnosis and detection
2 procedures. Further complicating the situation is the
3 very real possibility that a terrorist organization might
4 target either the food or water supply of a municipality
5 or even a nation itself, by attempting to include a
6 pathogenic microorganism or toxic contaminant capable of
7 causing widespread illness or even death. If, by accident
8 or design, the food supply of a particular population were
9 to be contaminated, it is not only imperative that the
10 population be alerted to the contamination, but it is
11 further necessary that the particular contaminant be
12 quickly and precisely pinpointed so that appropriate
13 countermeasures may be taken.

14 Thus, if it were possible to readily substitute
15 standard packaging materials with a flexible material
16 capable of
17 1) quickly and easily detecting the presence, and
18 2) indicating the particular identity of a variety of
19 pathogenic biological materials, a long felt need would be
20 satisfied.

21

22 **Description of the Prior Art**

23 The Berkeley Lab Research News of 12/10/96, in an
24 article entitle "New Sensor Provides First Instant Test
25 for Toxic E.Coli Organism" reports on the work of Stevens
26 and Cheng to develop sensors capable of detecting E. Coli
27 strain 0157:H7. A color change from blue to red
28 instantaneously signals the presence of the virulent E.
29 Coli 0157:H7 microorganism. Prior art required test
30 sampling and a 24 hour culture period in order to
31 determine the presence of the E. Coli microorganism,
32 requiring the use of a variety of diagnostic tools
33 including dyes and microscopes. An alternative technique,
34 involving the use of polymerase chain reaction technology,
35 multiplies the amount of DNA present in a sample until it

1 reaches a detectable level. This test requires several
2 hours before results can be obtained. The Berkeley sensor
3 is inexpensive and may be placed on a variety of materials
4 such as plastic, paper, or glass, e.g. within a bottle cap
5 or container lid. Multiple copies of a single molecule
6 are fabricated into a thin film which has a two part
7 composite structure. The surface binds the biological
8 material while the backbone underlying the surface is the
9 color-changing signaling system.

10 The Berkeley researchers do not teach the concept of
11 incorporating any means for self-detection within food
12 packaging, nor do they contemplate the inclusion of
13 multiple means capable of both detecting and identifying
14 the source of pathogenic contamination to a technically
15 untrained end user, e.g. the food purchaser or consumer.

16 Wang et al, in an article entitled "An immune-
17 capturing and concentrating procedure for Escherichia coli
18 0157:H7 and its detection by epifluorescence microscopy"
19 published in Food Microbiology, 1998, Vol. 15 discloses
20 the capture of E. coli on a polyvinylchloride sheet coated
21 with polyclonal anti-E. coli 0157:H7 antibody and stained
22 with fluorescein-labeled anti-E. coli 0157:H7. After
23 being scraped from the PVC surface, the cells were
24 subjected to epifluorescence microscopy for determining
25 presence and concentration. The reference fails to teach
26 or suggest the concept of incorporating any means for
27 self-detection within food packaging, nor does it
28 contemplate the inclusion of multiple means capable of
29 both detecting and identifying the source of pathogenic
30 contamination to a technically untrained end user, e.g.
31 the food purchaser or consumer, and especially fails to
32 disclose such detection without the use of specialized
33 detection techniques and equipment.

34 U.S. Patent 5,776,672 discloses a single stranded
35 nucleic acid probe having a base sequence complementary to

1 the gene to be detected which is immobilized onto the
2 surface of an optical fiber and then reacted with the gene
3 sample denatured to a single stranded form. The nucleic
4 acid probe, hybridized with the gene is detected by
5 electrochemical or optical detection methodology. In
6 contrast to the instantly disclosed invention, this
7 reference does not suggest the immobilization of the probe
8 onto a flexible polyvinylchloride or polyolefin film, nor
9 does it suggest the utilization of gelcoats having varying
10 porosities to act as a control or limiting agent with
11 respect to the migration of antibodies or microbial
12 material through the bioassay test material, or to serve
13 as a medium for enhancement of the growth of the microbial
14 material.

15 U.S. Patent 5,756,291 discloses a method of
16 identifying oligomer sequences. The method generates
17 aptamers which are capable of binding to serum factors and
18 all surface molecules. Complexation of the target
19 molecules with a mixture of nucleotides occurs under
20 conditions wherein a complex is formed with the specific
21 binding sequences but not with the other members of the
22 oligonucleotide mixture. The reference fails to suggest
23 the immobilization of the aptamers upon a flexible
24 polyvinylchloride or polyolefin base material, nor does it
25 suggest the use of a protective gelcoat layer which acts
26 as a means to selectively control the migration of
27 antibodies and antigens, or to serve as a medium for
28 enhancement of the growth of microbial material.
29

30 Summary of the Invention

31 The present invention relates to packaging materials
32 for food and other products, along with methods for their
33 manufacture and use. The presence of undesirable
34 biological materials in the packaged material is readily
35 ascertained by the consumer, merchant, regulator, etc.

1 under ordinary conditions and without the use of special
2 equipment. A multiplicity of biological materials
3 threaten our food supply. The present invention provides
4 a unique composite material capable of detecting and
5 identifying multiple biological materials within a single
6 package. The biological material identification system is
7 designed for incorporation into existing types of flexible
8 packaging material such as polyvinylchloride and
9 polyolefin films, and its introduction into the existing
10 packaging infrastructure will require little or no change
11 to present systems or procedures. Thus, the widespread
12 inclusion of the biological material detecting system of
13 the instant invention will be both efficient and
14 economical.

15 In one embodiment of the invention the biological
16 material detecting system prints a pattern containing
17 several antibodies or aptamers, derived from plant or
18 animal origins, onto a packaging material which is usually
19 a type of polymeric film, preferably a polyvinylchloride
20 or polyolefin film and most preferably a polyethylene film
21 which has undergone a surface treatment, e.g. corona
22 discharge to enhance the film's ability to immobilize the
23 antibodies upon its surface. The agents are protected by
24 a special abrasion resistant gel coat in which the
25 porosity is tailored to control the ability of certain
26 antibodies, toxic substances, etc. to migrate
27 therethrough. Each antibody is specific to a particular
28 biological material and is printed having a distinctive
29 icon shape. The detection system may contain any number
30 of antibodies capable of detecting a variety of common
31 toxic food microbes; although any number of microbes may
32 be identified via the inventive concept taught herein, for
33 the purpose of this description, the microbes of interest
34 will be limited to E.Coli, Salmonella, Listeria and
35 Cyclospora.

1 An important feature of the biological material
2 detection system is its all-encompassing presence around
3 and upon the product being packaged. Since the biological
4 material detecting system is designed as an integral part
5 of 100% of the packaging material and covers all surfaces
6 as utilized, there is no part of the packaged product
7 which can be exposed to undetected microbes. In the past,
8 the use of single location or *in situ* detectors have left
9 a majority of the area around and upon the packaged
10 product exposed to undetected microbes. This greatly
11 increased the chance that a spoiled or tainted product
12 might be inadvertently consumed before the toxic agent had
13 spread to the location of the *in situ* detector. The
14 biological material detection system of the present
15 invention avoids this problem by providing a plurality of
16 individual detectors per unit area which are effective to
17 insure positive detection of any pathogenic microorganisms
18 within the product being tested. In order to be effective
19 a particular degree of sensitivity is required, e.g. the
20 detecting system must be capable of positively identifying
21 one microbial cell in a 25 gram meat sample. In a
22 preferred embodiment, four detectors per square inch of
23 packaging material surface have been utilized, and in a
24 most preferred embodiment nine or more detectors per
25 square inch are incorporated upon the film's surface.

26 By use of the biological material detection system of
27 the present invention a packager or processor can
28 independently determine the multiplicity and identity of
29 those biological materials against which the packaged
30 product is to be protected. Although it is envisioned
31 that the large majority of biological material detection
32 treated packaging will be generic to approximately four of
33 the most common microbes, the system will nevertheless
34 allow each user to customize the protection offered to the
35 public.

1 The biological material detecting system will not
2 merely detect the presence of biological materials, it
3 will also identify the particular biological materials
4 located in a packaged product. This unique feature allows
5 for the immediate identification of each particular
6 biological material present since the antibodies are
7 specific to a detector having a definitive icon shape or
8 other identifying characteristic. Although the end use
9 consumer is primarily interested in whether a food product
10 is, or is not, contaminated per se, the ability to detect
11 and identify the particular biological material
12 immediately is of immeasurable value to merchants,
13 processors, regulators and health officials. The ability
14 to immediately identify a toxic material will lead to
15 greatly reduced response times to health threats that
16 might be caused by the biological material and will also
17 enhance the ability for authorities to locate the source
18 of the problem. The biological material detecting system
19 of the present invention exhibits an active shelf life in
20 excess of 1 year under normal operating conditions. This
21 enhances the use of a biological material detection system
22 on products which are intended to be stored for long
23 periods of time. If these products are stored so as to be
24 ready for immediate use in some time of emergency, then it
25 is extremely beneficial to definitely be able to determine
26 the safety of the product at the time that it is to be
27 used.

28 One particularly important feature of the biological
29 material detecting system of the instant invention is its
30 ability to quantitatively sensitize the reagents so as to
31 visually identify only those biological materials which
32 have reached a predetermined concentration or threshold
33 level which is deemed to be harmful to humans.

34 For example, almost all poultry meat contain traces
35 of the salmonella bacteria. In most cases, the salmonella

1 levels have not reached a harmful level of concentration.
2 The biological material detecting reagents are designed to
3 visually report only those instances where the level of
4 concentration of biological materials are deemed harmful
5 by health regulatory bodies.

6 The method of production of the biological material
7 detecting system is designed to be easily incorporated
8 within the packaging infrastructure of existing systems
9 without disruption of the systems or the procedures under
10 which they are operating. The biological material
11 detecting system can be incorporated onto packaging films
12 which are produced by the packager, or those which are
13 supplied by a film manufacturer. The apparatus necessary
14 for applying the biological material detecting system may
15 be easily located at the beginning of any continuous
16 process such as printing or laminating and will operate as
17 an integral part of an existing system.

18 The biological material detecting system of the
19 instant invention represents an entirely new packaging
20 material which is designed to inform the consumer of the
21 presence of certain biological materials or pathogens
22 present in food stuffs or other materials packaged within
23 the detecting system. The system is designed so that the
24 presence of a biological material is presented to the
25 consumer in a distinct, unmistakable manner which is
26 easily visible to the naked eye.

27 Recent outbreaks of E.Coli and other health hazards
28 have presented serious problems to the general population
29 and have raised concerns regarding the safety of the food
30 supply.

31 It is an objective of the present invention to
32 provide a biological material detecting system for
33 protecting the consumer by detecting and unmistakably
34 presenting to the untrained eye visual icons on the
35 packaging material which signify the presence of a number

1 of pathogens in the food stuff or other materials which
2 are at a level harmful to humans.

3 It is another objective of the instant invention to
4 provide a bioassay material wherein an antigen detecting
5 antibody system is immobilized upon the surface of a
6 flexible polyolefin film.

7 It is still another objective of the instant
8 invention to provide a bioassay material wherein an
9 antigen detecting antibody system is immobilized upon the
10 surface of a flexible polyvinylchloride film.

11 It is a further objective of the invention to provide
12 a biological material detecting system which is so similar
13 in appearance and utilization that its use, in lieu of
14 traditional packaging materials, is not apparent to the
15 food processor or other packagers.

16 A still further objective of the present invention is
17 to provide a biological material detecting system which is
18 cost effective when compared to traditional packaging
19 materials.

20 Other objectives and advantages of this invention
21 will become apparent from the following description taken
22 in conjunction with the accompanying drawings wherein are
23 set forth, by way of illustration and example, certain
24 embodiments of this invention. The drawings constitute a
25 part of this specification and include exemplary
26 embodiments of the present invention and illustrate
27 various objects and features thereof.

28

29 Brief Description of the Figures

30 Figure 1 is a cross-sectional interpretation of an
31 antibody sandwich immunoassay device;

32 Figure 2 is a cross-sectional interpretation of a single
33 ligand assay;

34 Figure 2A is a cross-sectional interpretation of a single
35 ligand assay including a chromogenic ligand;

1 Figure 3 is a diagrammatic representation showing the
2 functioning of a single ligand assay;
3 Figure 4 is a cross-sectional interpretation of an
4 antibody sandwich immunoassay including a scavenger system
5 for microbial quantification;
6 Figures 5 and 6 are a diagrammatic representation showing
7 the functioning of a sandwich assay/scavenger system;
8 Figure 7 is a planar view of an example of icon placement
9 and printing;
10 Figure 7A is an example of a typical code of
11 identification applied to the icon pattern;
12 Figure 8 is the result derived from EXAMPLE 2 and
13 exemplifies capture sensitivity of a single ligand treated
14 polyvinylchloride film;
15 Figure 9 is a block diagram of the apparatus illustrating
16 the process steps for forming a sandwich assay;
17 Figure 10 is a block diagram of the apparatus illustrating
18 the process steps for forming a single ligand assay.
19

20 **Description of the Preferred Embodiment(s)**

21 Referring now to Figure 1, the detection and
22 identification of various biological materials in packaged
23 foods or other products is accomplished by the use of
24 antibodies which are specific to the biological material
25 being sought. Specific antibodies, defined as capture
26 antibodies, are biologically active ligands characterized
27 by their ability to recognize an epitope of the particular
28 toxic substance being tested for. These capture
29 antibodies are selected from such materials as antibodies,
30 aptamers, single stranded nucleic acid probes, lipids,
31 natural receptors, lectins, carbohydrates and proteins.
32 In one embodiment of the invention, the capture antibodies
33 are arranged with unique icon shapes and in particular
34 patterns. The capture antibodies are immobilized to the
35 polymer film. An agarose gel coat containing detector

1 antibodies is printed in register above the capture
2 antibodies. A protective gel coat completes the
3 construction of the packaging material. The gel coat
4 constituting the inner layer, e.g. that layer which is
5 next to the packaged product, is a special type of gel
6 coat or an equivalent thereto which has sufficient
7 porosity to allow toxic molecules, known as antigens, to
8 migrate through it to an antibody "sandwich" laminated
9 between the polymer film and the gel coat. The special
10 gel coat has sufficient abrasion resistance to prevent
11 exposure of the reagents to the product. The special gel
12 coat useful in the invention is a readily available
13 coating commonly utilized in the food industry to coat
14 candies and the like, e.g. coated chocolates to prevent
15 them from melting on one's hands. Migration of antigens
16 is driven by capillary action and normally reaches a state
17 of equilibrium within a 72 hour time period. In a
18 particularly preferred embodiment, when operating within a
19 temperature range of 4 - 25 degrees Celsius, an initial
20 positive reading can be obtained within 30 minutes, and
21 the test continues to yield results for about 72 hours.
22 Upon migrating through the special gel coat the antigen
23 enters an agarose gel film which has surfactant
24 properties, contains free detector antibodies, and also
25 contains one or more ingredients designed to enhance the
26 growth of microbial materials, e.g. nutrients such as
27 sorbitol, NOVOBIOCIN, CEFIXIME and TELLURITE which
28 increase the growth rate and ease isolation of E. Coli
29 0157H. If the antigen encounters a species of antibody
30 which is specific to an epitope thereof, it will then bind
31 to it forming a detector/antibody complex. Once bound
32 thereto, the bound antigen/antibody complex becomes too
33 large to migrate back through the special gel coat due to
34 its inherent fine porous structure. This insures that
35 pathogenic material can not migrate back into the product

1 being tested. Continuing pressure toward equilibrium from
2 capillarity will tend to move the antigen, with its bound
3 antibody, through a second gel coat layer and into an area
4 of the flexible polyvinylchloride or polyolefin film
5 containing corresponding species of immobilized capture
6 antibodies. The layer of immobilized antibodies is
7 attached to the outer polymer film in predetermined
8 patterns of simple icons, as best seen in Figures 7,7A.
9 When the particular species of bound antigen encounters a
10 particular corresponding species of immobilized antibody
11 specific to a separate and distinct epitope thereof,
12 further binding occurs. Upon the antigen binding to the
13 two antibodies, a distinct icon shape emerges on the outer
14 film at the point of binding, thereby providing a visual
15 indicator.

16 While it is theoretically possible to detect an
17 unlimited number of pathogens present in a packaged
18 product, then to present this information in a very clear
19 and unmistakable manner to an untrained consumer, as a
20 practical matter there are limits to the amount of
21 information which can be developed and presented in the
22 biological material detecting system. Some of the
23 limiting factors are cost, available surface area for
24 display of information, complexity, and other
25 considerations. Thus, for illustrative purposes only, the
26 biological material detecting system as exemplified herein
27 utilizes four separate pairs of antibodies, as set forth
28 in Figures 7 and 7A. This is in no way meant to suggest a
29 limit on the number of antibodies that can be utilized, in
30 a single biological material detecting system.
31 As demonstrated in Figures 7 and 7A, the invention is
32 exemplified with reference to detection of the following
33 four microbes:
34 1. E-Coli;
35 2. Salmonella;

- 1 3. Listeria; and
- 2 4. Cyclospora.

3 To each of the four microbes, a particular icon shape
4 is assigned. Although there are infinite numbers of icons
5 which might be used including letters, numbers, or even
6 words, we have chosen simple identifiers for the purpose
7 of demonstration. As an initial step in the construction
8 of the biological material detecting system, the outer
9 polymer film or base layer undergoes a printing process in
10 which a pattern of the four icons, wherein each icon
11 utilizes a specific species of immobilized capture
12 antibody, is applied thereto. Corresponding species of
13 free antibodies, known as detector antibodies, which are
14 biologically active ligands characterized by their ability
15 to recognize a different epitope of the same particular
16 toxic substance being tested for, and suspended in an
17 agarose gel solution containing a surfactant and a
18 nutrient, are printed in registration with the immobilized
19 antibodies so as to be in overlying and juxtaposed
20 relationship thereto, and are then dried. Lastly, a
21 second gel coat having a degree of porosity sufficient to
22 prevent passage of the detector antibodies is laminated to
23 the preparation.

24 Although the detection of biological materials
25 through the use of antibodies is well known, there are
26 several new and novel aspects to the application of
27 antibody science which are set forth in the development of
28 the biological material detecting system of the present
29 invention.

30 Among these are: 1) the use of multiple antibodies to
31 detect multiple biological materials in individual
32 packages; 2) the use of a distinctive icon or other shape
33 to not only detect, but visually identify the biological
34 materials to the consumer, vendor, regulator, etc.;
35 3) insuring that detection and identification of the

1 biological materials is accomplished in a timely manner in
2 each particular application by judiciously controlling the
3 porosity of the gel coat, thereby controlling the lapse
4 rate of the reaction through the strength of capillary
5 action; 4) inclusion of additives within the special gel
6 coat to enhance the levels of microbes present; 5)
7 incorporating the biological material detecting system of
8 the instant invention within the existing packaging
9 industry infrastructure; and 6) providing a bioassay
10 material and methods for its production and use which
11 immobilizes the antibodies onto the surface of a flexible
12 polyvinylchloride or polyolefin, e.g. a polyethylene, a
13 surface treated polyethylene, a polypropylene, a surface
14 treated polypropylene or mixture thereof.

15 The embodiment discussed above is based upon a
16 sandwich immunoassay as depicted in Figure 1, which
17 measures specific microbes, wherein the particular toxic
18 substance is one or more members selected from the group
19 consisting of a particular microorganism, biological
20 materials containing the genetic characteristics of said
21 particular microorganism, and mutations thereof. In a
22 particular embodiment, the toxic substance is selected
23 from the group consisting of microorganisms, nucleic
24 acids, proteins, integral components of microorganisms and
25 combinations thereof.

26 It should also be understood that the invention will
27 function by direct measurement of microbes with certain
28 types of antibodies, selected from the group consisting of
29 an antibody, a single stranded nucleic acid probe, an
30 aptamer, a lipid, a natural receptor, a lectin, a
31 carbohydrate and a protein. The biological materials may
32 also be measured by non-immunological methods in
33 particular using labeled molecules, such as aptamers,
34 which have a high affinity for the biological materials.

1 The invention utilizes various types of detector
2 antibodies, e.g. those conjugated with dyes to produce a
3 visual cue, or alternatively, photoactive compounds
4 capable of producing a visual cue in response to a
5 particular type of light exposure, for example a scanning
6 system which detects luminescent properties which are
7 visualized upon binding of the antigen and antibody. In
8 this method of construction biological materials are
9 measured directly with a biologically active ligand, e.g.
10 an antibody, aptamer, nucleic acid probe or the like,
11 which induces a conformational change to produce a visual
12 cue.

13 It is also understood that specific polymers may be
14 incorporated into the invention and that when a biological
15 material is bound to the surface it induces a molecular
16 change in the polymer resulting in a distinctly colored
17 icon. Referring to Figures 2 and 2A, in an alternative
18 embodiment a sandwich-type of construction is not
19 necessary. As depicted in Figures 2 and 2A, the provision
20 of certain types of biologically active ligand, e.g.
21 chromogenic ligands to which receptors are bound will
22 permit the visual confirmation of binding of the antigen
23 to the immobilized ligand.

24 As depicted in Figure 3, a polymer film is provided
25 and a biologically active ligand, preferably a chromogenic
26 ligand, is immobilized to the polymer film. In the past,
27 immobilized ligands were attached to rigid solid support
28 matrices such as plastic, polystyrene beads, microtitre
29 plates, latex beads, fibers, metal and glass surfaces and
30 the like. The immobilized ligands have also been attached
31 to flexible surfaces such as nitrocellulose or polyester
32 sheets which were not transparent. Surprisingly, the
33 inventor has discovered that it is possible to attach
34 biologically active ligands to the surface of various
35 flexible polymeric films, for example polyvinylchloride

1 and polyolefins, e.g. a polyolefin sheet having
2 appropriate properties of transparency and flexibility and
3 that the composite functions as a biological sensor or
4 assay material. After printing on the polymer film, the
5 material goes through a drying step; subsequent to which a
6 special gel coat or liquid film is applied as a protectant
7 layer and the final product is then dried.

8 Illustrative of films which will function in the
9 present invention is a film containing a structural
10 polymer base having a treated surface and incorporating
11 therein a fluorescing antibody receptor and finally a
12 stabilized gel coat. These films may be untreated
13 polyethylene or polyvinylchloride films which are amenable
14 to antibody immobilization by various mechanisms, e.g. by
15 adsorption. In a particular embodiment, the films may be
16 first cleaned, e.g. by ultrasonication in an appropriate
17 solvent, and subsequently dried. For example the polymer
18 sheet may be exposed to a fifteen minute ultrasonic
19 treatment in a solvent such as methylene chloride,
20 acetone, distilled water, or the like. In some cases, a
21 series of solvent treatments are performed. Subsequently
22 the film is placed in a desiccating device and dried.
23 Alternatively, these films may be created by first
24 exposing the film to an electron discharge treatment at
25 the surface thereof, then printing with a fluorescing
26 antibody receptor. Subsequently, a drying or heating step
27 may be utilized to treat the film to immobilize the
28 receptor. Next, the film is washed to remove un-
29 immobilized receptor; the film is then coated with a gel
30 and finally dried.

31 Additional modifications to polyolefin films may be
32 conducted to create the presence of functional groups, for
33 example a polyethylene sheet may be halogenated by a free
34 radical substitution mechanism, e.g. bromination,
35 chlorosulfonation, chlorophosphorylation or the like.

1 Furthermore, a halodialkylammonium salt in a sulfuric acid
2 solution may be useful as a halogenating agent when
3 enhanced surface selectivity is desirable.

4 Grafting techniques are also contemplated wherein
5 hydrogen abstraction by transient free radicals or free
6 radical equivalents generated in the vapor or gas phase is
7 conducted. Grafting by various alternative means such as
8 irradiation, various means of surface modification,
9 polyolefin oxidation, acid etching, inclusion of chemical
10 additive compounds to the polymer formulation which have
11 the ability to modify the surface characteristics thereof,
12 or equivalent techniques are all contemplated by this
13 invention.

14 Additionally, the formation of oxygenated surface
15 groups such as hydroxyl, carbonyl and carboxyl groups via
16 a flame treatment surface modification technique is
17 contemplated.

18 Further, functionalization without chain scission by
19 carbene insertion chemistry is also contemplated as a
20 means of polyolefin polymer modification.

21 Illustrative of the types of commercially available
22 films which might be utilized are polyvinylchloride films
23 and a straight polyethylene film with electron discharge
24 treatment marketed under the trademark SCLAIR®. The
25 electron discharge treatment, when utilized, renders the
26 film much more susceptible to immobilization of the
27 antibodies on its surface. Additional films which might
28 be utilized are Nylon 66 films, for example DARTEK®, a
29 coextrudable adhesive film such as BYNEL® and a blend of
30 BYNEL® with polyethylene film.

31 With reference to Figures 4-6, one of the most
32 important features of the biological material detecting
33 system is its ability to quantitatively sensitize the
34 antibody or aptamer so as to visually identify only those
35 biological materials that have reached a concentration

1 level deemed harmful to humans. One means of providing
2 this sensitization is by including a scavenger antibody
3 which is a biologically active ligand characterized as
4 having a higher affinity for the particular toxic
5 substance than the capture antibody. The scavenger
6 antibody is provided in a sufficient amount to bind with
7 the particular toxic substance up to and including a
8 specific threshold concentration. In this manner, the
9 capture antibody will be prevented from binding with a
10 detector antibody until the concentration of the
11 particular biological material surpasses the specific
12 threshold concentration. In this manner, the biological
13 material detecting system visually reports only those
14 instances where concentration levels are deemed harmful by
15 health regulatory bodies.

16 Since the biological material detecting system as
17 described herein can maintain its activity over long
18 periods of time, e.g. up to 1 year, it is able to protect
19 against contamination in products which have long shelf
20 lives. Additionally, by reporting only toxic
21 concentrations, it avoids "false positives" and, in some
22 cases, can extend the useful life of the product.

23 Referring to Figures 9 and 10, the apparatus for
24 producing the biological material detecting system is
25 illustrated. These embodiments are essentially particular
26 combinations of printers, coaters and dryers which will be
27 used to place biologically active reagents upon a thin
28 polymer film useful for packaging food stuffs and other
29 products. These films will be further processed
30 subsequent to application of the biological material
31 detecting system by printing, laminating, or equivalent
32 methods of fabrication. The machinery is designed so that
33 it will transport and process very thin films at rather
34 high speeds. Furthermore, the machinery is designed so
35 that it can be utilized effectively as an additional

1 processing step when added to continuous processing
2 operations already in use at packaging material
3 fabrication plants. The printing machinery is designed so
4 that a minimum of four distinct biological active ligands
5 in a hydrate solution can be printed in patterns in a
6 precise registration on the polymer film. The printing
7 may be accomplished by jet spray or roller application, or
8 equivalent printing methods. Each print applicator is
9 capable of printing a detailed icon no larger than 1/4" x
10 1/4" in a minimum thickness. Patterning may be controlled
11 by computer or roller calendaring. It is important to
12 determine the appropriate viscosity of the solution to be
13 applied so that successful printing, coating, and drying
14 can be accomplished. After the printing step the icons
15 must be protected. This is accomplished by a final
16 application of a thin special gel coat or a thin liquid
17 film. This step is accomplished by a 100% coating of the
18 entire film or alternatively by selectively coating each
19 icon such that a 10% overlap is coated beyond the icon in
20 all directions. This coating step may be accomplished
21 with sprays or rollers and the viscosity of the coating
22 material must be optimized so as to provide adequate
23 coverage. The biological material detecting system must
24 be dried after printing and once again after coating. The
25 drying is accomplished in a very rapid manner so as to
26 enable high through put for the process. Various means of
27 drying include the use of radiant heat, convected air and
28 freeze drying. Care must be taken to avoid drying
29 temperatures which will inactivate the biological reagents
30 which have been applied. The polymer film which has been
31 surface treated in the form of electron discharge, e.g.
32 corona treatment, is most preferred. After preparation,
33 the thin film is transported at relatively high speeds so
34 that a wrinkle free surface is provided for printing,
35 coating and rollup. Additionally, the apparatus provides

1 a complete recovery system for the reagents which allows
2 for total recovery of the agents and the volatile organic
3 contaminants.

4 The invention will be further illustrated by way of
5 the following examples:

6 **EXAMPLE 1**

7 **Detection of Antibody on the Surface of a Thin Layer**

8 **Polyvinylchloride Sheet:**

9 Rabbit polyclonal IgG was diluted to a final concentration
10 of 2.0 µg/ml in 0.1M carbonate (Na_2CO_3) -bicarbonate
11 (NaHCO_3) buffer, pH 9.6.

12 Using a 2" x 3" grid, 75 µL (150 ng) was applied to a
13 sheet of polyvinylchloride at 1"intervals.

14 The antibody treated polyvinylchloride sheet was dried for
15 1.5 hrs. at a temperature of 37°C.

16 The dried sheet was then washed 3 times with a phosphate
17 buffered saline solution at a ph of 7.4.

18 HRP conjugated goat anti-rabbit IgG ($\text{G}\alpha\text{R}^{\text{HRP}}$) was diluted to
19 a concentration of 1:7000 in 1% casein, 0.1M potassium
20 ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$, 0.1% phosphate glass ($\text{Na}_{15}\text{P}_{13}\text{O}_{40}$ -
21 $\text{Na}_{20}\text{P}_{18}\text{O}_{55}$), at a pH of 7.4.

22 A precision pipette was used to apply 125 µL of diluted
23 G^{HRP} to the grid backed polyvinylchloride sheet at 1"
24 intervals coinciding with the area covered by the
25 previously coupled RαG.

26 The sheet was incubated at room temperature for 30
27 minutes.

28 The sheet was then washed 3X with phosphate buffered
29 saline at a pH of 7.4.

30 125µL of precipitating TMB enzyme substrate was added to
31 the test areas.

32 The sheet was incubated at room temperature until color
33 development was complete.

34 Lastly the sheet was washed 3 times with deionized water
35 and allowed to air dry.

1 **EXAMPLE 2**2 **Full Sandwich Immunoassay on the Surface of a Thin Layer**
3 **Polyvinylchloride Sheet**

4

5 Rabbit polyclonal IgG was diluted to a final
6 concentration of 2.0 µg/ml in 0.1M carbonate (Na_2CO_3) -
7 bicarbonate (NaHCO_3) buffer, pH 9.6.

8 A 13 x 9 cm piece of thin layered polyvinylchloride
9 sheet was inserted into a BIO-RAD DOT-SPOT apparatus
10 possessing 96 sample wells spaced at 1.0 cm intervals in a
11 12 x 8 well grid.

12 A 100 µL sample (1.0 µg) of rabbit polyclonal IgG was
13 applied to each well 8 of column 1.

14 Antibody samples applied to columns 2-12 represented
15 serial dilutions of the antibody ranging from 500 ng - 0.5
16 ng.

17 The antibody treated polyvinylchloride sheet was
18 dried overnight at 37° C.

19 The dried sheet was washed 3 times with phosphate
20 buffered saline (PBS), pH 7.4.

21 Antigen was diluted to a final concentration of 1.0
22 µg/ml in tris buffered saline (TBS) with 1% casein, pH
23 7.4.

24 100 µL, representing 100 ng, of antigen, was applied
25 to each well of the apparatus and incubated at room
26 temperature for 1 hour.

27 The polyvinylchloride sheet was washed 3 times with
28 phosphate buffered saline (PBS), pH 7.4.

29 Detector mouse monoclonal antibody was diluted was
30 diluted 1:625 with TBS containing 1% casein, 0.1M
31 potassium ferricyanide $\text{K}_3\text{Fe}(\text{CN})_6$, and 0.1% phosphate glass
32 ($\text{Na}_{15}\text{P}_{13}\text{O}_{40}$ - $\text{Na}_{20}\text{P}_{18}\text{O}_{55}$), pH 7.4.

33 100 µL of the 1:625 dilution of detector antibody
34 solution was applied to each well of row # 1.

35 Detector samples of 100 µL applied to rows 2-7

1 represented serial dilutions of the antibody ranging from
2 1:1,250 to 1:80,000. Dilutions of detector antibody were
3 incubated on the polyvinylchloride sheet for 1 Hr. at room
4 temperature.

5 The polyvinylchloride sheet was washed 3 times with
6 phosphate buffered saline (PBS), pH 7.4.

7 100 µL of goat anti-mouse IgG^{HRP} were added to each
8 well of the DOT-SPOT apparatus and allowed to incubate for
9 one hour at room temperature.

10 The polyvinylchloride sheet was washed 3 times with
11 phosphate buffered saline (PBS), pH 7.4.

12 100 µL of precipitating TMB enzyme substrate was
13 added to the test areas.

14 The sheet was incubated at room temperature until
15 color development was complete (see Figure 8).

16 Lastly the sheet was washed 3 times with deionized
17 water and allowed to air dry.

18 It is to be understood that while a certain form of
19 the invention is illustrated, it is not to be limited to
20 the specific form or arrangement of parts herein described
21 and shown. It will be apparent to those skilled in the
22 art that various changes may be made without departing
23 from the scope of the invention and the invention is not
24 to be considered limited to what is shown in the drawings
25 and described in the specification.

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CLAIMS

7 What is claimed is:

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9 Claim 1. A biological assay material for detecting
10 the presence of a particular toxic substance comprising:11 a base layer which is a flexible film for
12 immobilization of a ligand applied to a surface thereof
13 and wherein said film is selected from the group
14 consisting of a polyolefin or a polyvinylchloride;15 a capture antibody which is a biologically active
16 ligand characterized by its ability to recognize an
17 epitope of the particular toxic substance, said ligand
18 being immobilized onto said surface of said film;19 a first agarose gelcoat layer overlying the capture
20 antibody, said agarose layer being permeable to the toxic
21 substance and containing at least one ingredient to
22 enhance the growth thereof, said layer further containing
23 a detector antibody which is a biologically active ligand
24 characterized by its ability to recognize a different
25 epitope of said particular toxic substance, thereby
26 forming a detector antibody/antigen complex; and27 a second protective gelcoat layer overlying the
28 detector antibody and having a degree of porosity whereby
29 passage of said toxic substance is permitted and passage
30 of said detector antibody/antigen complex is prevented,
31 said second protective gelcoat layer having a degree of
32 abrasion resistance effective to protect the biological
33 assay material.

34

35

1 Claim 2. The biological assay material according to
2 claim 1 wherein the flexible film is a polyolefin selected
3 from the group consisting of polyethylene, polypropylene
4 and mixtures thereof.

5

6 Claim 3. The biological assay material according to
7 claim 1 wherein the flexible film is a polyvinylchloride.

8

9 Claim 4. The biological assay material according to
10 claim 1 wherein the particular toxic substance is one or
11 more members selected from the group consisting of a
12 particular microorganism, biological materials containing
13 the genetic characteristics of said particular
14 microorganism, and mutations thereof.

15

16 Claim 5. The biological assay of material according
17 to claim 1 wherein the particular toxic substance is
18 selected from the group consisting of microorganisms,
19 nucleic acids, proteins, integral components of
20 microorganisms and combinations thereof.

21

22 Claim 6. The biological assay material according to
23 claim 1 wherein the ligand is selected from the group
24 consisting of an antibody, a single stranded nucleic acid
25 probe, an aptamer, a lipid, a natural receptor, a lectin,
26 a carbohydrate and a protein.

27

28 Claim 7. The biological assay material according to
29 claim 1 further including a scavenger antibody which is a
30 biologically active ligand characterized as having a
31 higher affinity for the particular toxic substance than
32 the capture antibody, said scavenger antibody being
33 present in a sufficient amount to bind with the particular
34 toxic substance up to and including a specific threshold
35 concentration;

1 whereby a capture antibody will be prevented from
2 binding with a detector antibody until the concentration
3 of the particular biological material surpasses the
4 specific threshold concentration.

5

6 Claim 8. A method to detect the presence or absence
7 of a particular toxic substance, which method comprises:

8 a) providing a base layer which is a flexible film
9 for immobilization of a ligand applied to a surface
10 thereof and wherein said film is selected from the group
11 consisting of a polyolefin or a polyvinylchloride;
12 b) providing a capture antibody which is a
13 biologically active ligand characterized by its ability to
14 recognize an epitope of the particular toxic substance,
15 said ligand being immobilized onto said surface of said
16 flexible film;

17 c) providing a first agarose gelcoat layer overlying
18 the capture antibody, said agarose layer being permeable
19 to the toxic substance and containing at least one
20 ingredient to enhance the growth of the toxic substance,
21 said layer further containing a detector antibody which is
22 a biologically active ligand characterized by its ability
23 to recognize a different epitope of said particular toxic
24 substance;

25 d) providing a second protective gelcoat layer
26 overlying the detector antibody and having a degree of
27 porosity sufficient to prevent passage of said detector
28 antibody therethrough;

29 e) placing said biological assay material in an
30 environment which may contain a particular toxic
31 substance; and

32 f) monitoring said biological assay material for a
33 period of time sufficient to observe a visual signal which
34 will confirm the presence or absence of the particular
35 toxic substance.

1

2 Claim 9. A material useful for food packaging and
3 characterized by its ability to detect the presence and
4 particularly identify one or more toxic substances
5 comprising:

6 a base layer which is a flexible film for
7 immobilization of a ligand applied to a surface thereof
8 and wherein said film is selected from the group
9 consisting of a polyolefin or a polyvinylchloride;

10 a capture antibody which is a biologically active
11 ligand characterized by its ability to recognize an
12 epitope of the particular toxic substance, said ligand
13 being immobilized onto said surface of said flexible film;

14 a first protective agarose gelcoat layer overlying
15 the capture antibody, said agarose layer being permeable
16 to the toxic substance;

17 a detector antibody which is a biologically active
18 ligand characterized by its ability to recognize a
19 different epitope of said particular toxic substance, said
20 detector antibody overlying said first protective gelcoat
21 layer and in juxtaposed relation thereto; and

22 a second gelcoat layer overlying the detector
23 antibody and having a degree of porosity sufficient to
24 prevent passage of said detector antibody therethrough.

25

26 Claim 10. The material according to claim 9 wherein
27 the flexible polyolefin film is selected from the group
28 consisting of polyethylene, polypropylene and mixtures
29 thereof.

30

31 Claim 11. The material according to claim 9 wherein
32 the flexible film is a polyvinylchloride.

33

34 Claim 12. The material according to claim 9 wherein
35 the particular toxic substance is one or more members

1 selected from the group consisting of a particular
2 microorganism, biological materials containing the genetic
3 characteristics of said particular microorganism, and
4 mutations thereof.

5

6 Claim 13. The material according to claim 9 wherein
7 the particular toxic substance is selected from the group
8 consisting of microorganisms, nucleic acids, proteins,
9 integral components of microorganisms and combinations
10 thereof.

11

12 Claim 14. The material according to claim 9 wherein
13 the ligand is selected from the group consisting of an
14 antibody, a single stranded nucleic acid probe, an
15 aptamer, a lipid, a natural receptor, a lectin, a
16 carbohydrate and a protein.

17

18 Claim 15. The material according to claim 9 further
19 including a scavenger antibody which is a biologically
20 active ligand characterized as having a higher affinity
21 for the particular toxic substance than the capture
22 antibody, said scavenger antibody being present in a
23 sufficient amount to bind with the particular toxic
24 substance up to and including a specific threshold
25 concentration;

26 whereby a capture antibody will be prevented from
27 binding with a detector antibody until the concentration
28 of the particular biological material surpasses the
29 specific threshold concentration.

30

31 Claim 16. The material according to claim 9 wherein
32 one or more species of capture antibody are
33 immobilized onto said surface of said flexible film in a
34 particular orientation, each of said one or more species
35 being characterized by a unique shape; and

1 one or more corresponding species of detector
2 antibody are applied onto the surface of said first
3 protective gelcoat layer in the same particular
4 orientation as said one or more species of capture
5 antibody, each of said one or more species being
6 characterized by a corresponding unique shape;

7 whereby simultaneous binding of any of the one or
8 more species of capture antibodies and one or more
9 corresponding species of detector antibodies with the
10 particular toxic substance which they recognize results in
11 the appearance of a visual signal having the unique shape
12 assigned to that species;

13 wherein an observer is alerted to the presence and
14 identity of said particular toxic substance.

15

16 Claim 17. A biological assay material for detecting
17 the presence of a particular toxic substance comprising:

18 a base layer which is a flexible film for
19 immobilization of a ligand applied to a surface thereof
20 and wherein said film is selected from the group
21 consisting of a polyolefin or a polyvinylchloride;

22 a biologically active ligand immobilized to the
23 flexible film; and

24 a gel coat or liquid film applied as a protectant
25 layer;

26 whereby binding of the particular toxic substance and
27 biologically active ligand produces a visual signal which
28 is indicative of both the presence and identity of said
29 particular toxic substance.

30

31 Claim 18. The biological assay material according to
32 claim 17 wherein the biologically active ligand is a
33 chromogenic ligand.

34

35

1 Claim 19. The biological assay material according to
2 claim 17 wherein the base layer is a film incorporating
3 thereon a fluorescing antibody receptor.

4

5 Claim 20. The biological assay material according to
6 claim 19 wherein the base layer is created by printing
7 with a fluorescing antibody receptor and drying or heating
8 the film to immobilize said receptor.

9

10 Claim 21. The biological assay material according to
11 claim 17 wherein a scavenger antibody which is a
12 biologically active ligand characterized as having a
13 higher affinity for the particular toxic substance than
14 the immobilized ligand is provided in a sufficient amount
15 to bind with the particular toxic substance up to and
16 including a specific threshold concentration;

17 whereby the assay material is quantitatively
18 sensitized so as to visually identify only those
19 particular toxic substances that have reached a
20 concentration level deemed harmful to humans.

21

22 Claim 22. The biological assay material according to
23 claim 18 wherein the chromogenic ligand is selected from
24 the group consisting of those conjugated with dyes to
25 produce a visual cue and those characterized as
26 photoactive compounds capable of producing a visual cue in
27 response to a particular type of light exposure;

28 whereby binding of the particular toxic substance and
29 chromogenic ligand results in a color change or
30 visualization of a luminescent property which is
31 indicative of both the presence and identity of said
32 particular toxic substance.

33

34

1 Claim 23. The biological assay material according to
2 claim 17 wherein the material is a food packaging
3 material.

4

5 Claim 24. The biological assay material according to
6 claim 17 containing a plurality of biologically active
7 ligands, each of said ligands being receptive to an
8 epitope of a different particular toxic substance and
9 having a unique shape;

10 whereby upon binding with one or more of said
11 different particular toxic substances, a visual signal
12 will result thereby alerting an observer to the presence
13 and identity of any or all of the particular toxic
14 substances to which said material is receptive.

15

16 Claim 25. The biological assay material according to
17 claim 17 wherein the particular toxic substance is one or
18 more members selected from the group consisting of a
19 particular microorganism, biological materials containing
20 the genetic characteristics of said particular
21 microorganism, and mutations thereof.

22

23 Claim 26. The biological assay of material according
24 to claim 17 wherein the particular toxic substance is
25 selected from the group consisting of microorganisms,
26 nucleic acids, proteins, integral components of
27 microorganisms and combinations thereof.

28

29 Claim 27. The biological assay material according to
30 claim 17 wherein the ligand is selected from the group
31 consisting of an antibody, a single stranded nucleic acid
32 probe, an aptamer, a lipid, a natural receptor, a lectin,
33 a carbohydrate and a protein.

34

35

1 Claim 28. The material according to claim 17 wherein
2 the flexible polyolefin film is selected from the group
3 consisting of polyethylene, polypropylene and mixtures
4 thereof.

5

6 Claim 29. The material according to claim 17 wherein
7 the flexible film is a polyvinylchloride.

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FIG. 1

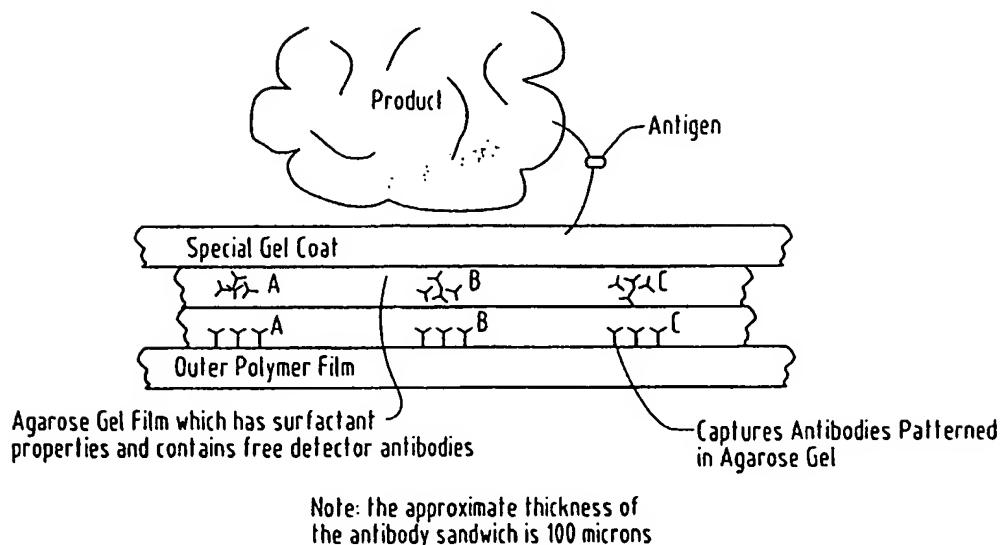


FIG. 2

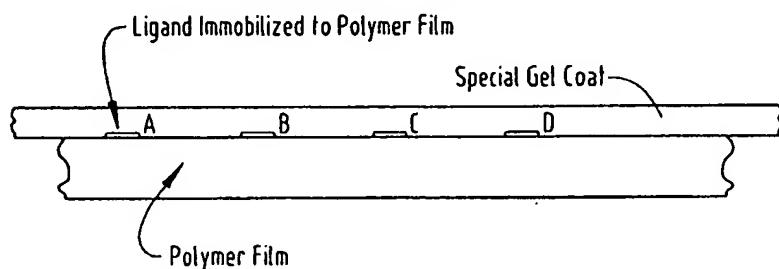
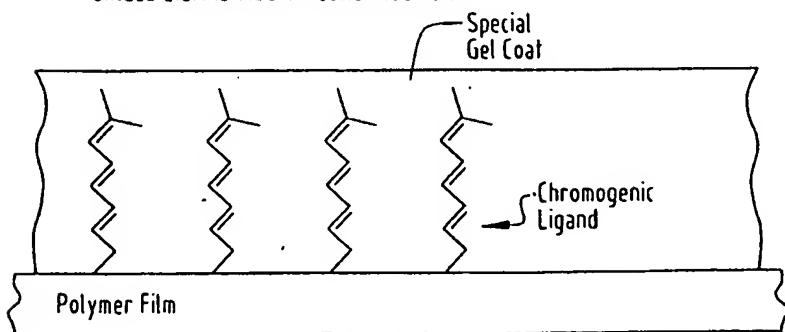


FIG. 2A

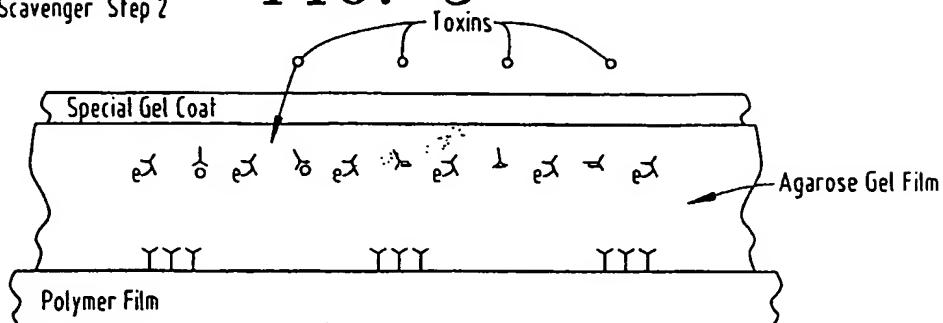
SINGLE LIGAND ASSAY CONSTRUCTION



A chromogenic ligand is immobilized on the polymer film in patterns of icons, and is coated with a porous gel which will allow the migration of toxins to the ligand.

Scavenger Step 2

FIG. 5



When toxins enter the sandwich, they will bind first with the unlabeled detector antibodies until all of these are bound.

FIG. 6

Scavenger Step 3

After all of the high affinity unlabeled detector antibodies are bound to the toxins, the detector antibodies labeled with a colorimeric enzyme will begin to bind to the toxins. The labeled complex will then begin to bind to the capture antibodies, producing a visual cue.

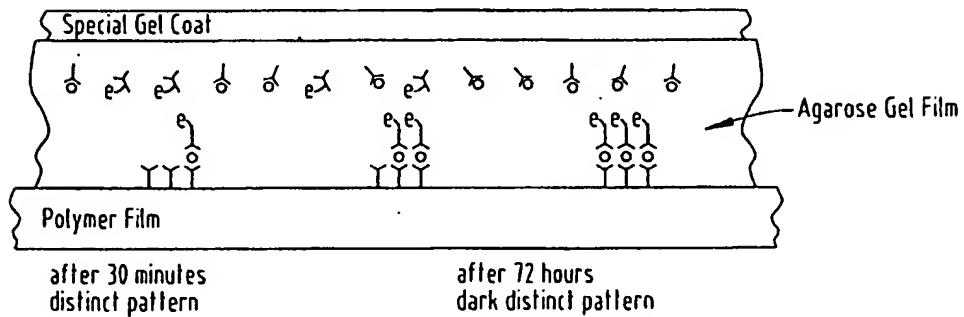


FIG. 7

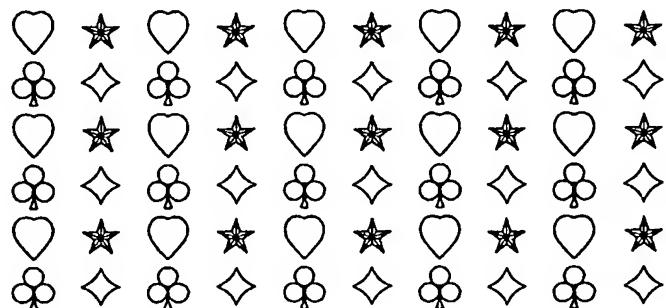
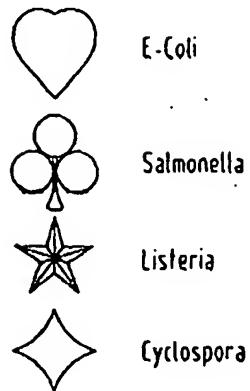
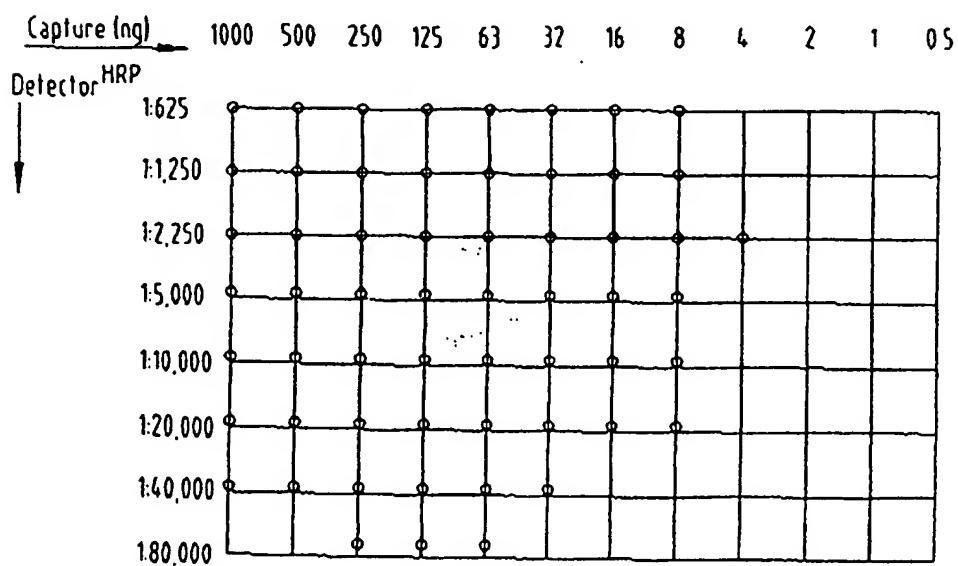
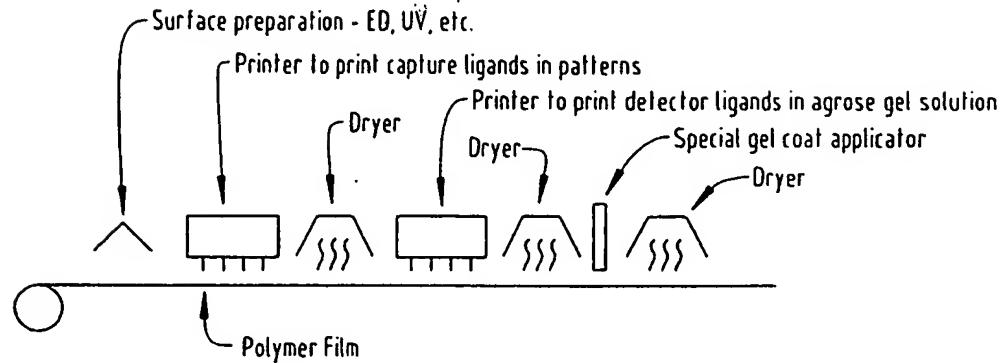


FIG. 7A**FIG. 8**

Checkerboard Dot-Spot Application of RaMBP on a Polyvinylchloride Surface and Detection by GaR^{HRP}



BEST AVAILABLE COPY**FIG. 9****FIG. 10**

GENERAL LAYOUT APPLICATION MACHINERY

